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3 **Different responses in leaf-level physiology to competition and facilitation**  
4 **under different soil types and N fertilization**

5  
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**Abstract** Knowledge of how competition and facilitation affect photosynthetic traits and nitrogen metabolism contributes to understanding of plant-plant interaction mechanisms. We transplanted two larch species, *Larix kaempferi* and *L. olgensis*, to establish intra- and interspecific interaction experiments under different types of soil. Experiment 1: Two different soil types were selected, one from a c. twenty years old *L. kaempferi* plantation (named larch soil) and another from a secondary natural forest (named mixed forest soil). The experiment included three types of plant interactions (*L. kaempferi* + *L. kaempferi*, *L. olgensis* + *L. olgensis*, and *L. kaempferi* + *L. olgensis*) and two soil types. Experiment 2: N fertilization was applied to larch soil. The experiment included the same three types of plant interactions as in Experiment 1 and two N treatments. The growth of *L. kaempferi* was negatively affected by larch soil and accelerated by N fertilization, particularly under interspecific interaction. The effects of soil type combined with plant-plant interactions or N fertilization influenced the chlorophyll pigment content, net photosynthetic rate ( $P_n$ ), photosynthetic N use efficiency (PNUE) and total non-structural carbohydrates of leaves (TNC).  $Chl\ a/Chl\ b$  (ratio of chlorophyll a to chlorophyll b) was higher when the growth of *L. kaempferi* was facilitated by the presence of *L. olgensis* in mixed forest soil. However, the ratio significantly declined when *L. kaempferi* confronted strong competition from *L. olgensis* in larch soil without N fertilization. Under N fertilization in larch soil,  $Chl\ a/Chl\ b$  of *L. olgensis* significantly increased by the presence of *L. kaempferi*. Plant-plant interactions and soil types affected the number of chloroplasts, especially in *L. kaempferi*, which had a greater number of chloroplasts under interspecific

1 interactions than in monoculture when growing in mixed forest soil. *L. olgensis*  
2 enhanced its ability to absorb N-NO<sub>3</sub><sup>-</sup> under interspecific interactions in larch N- soil,  
3 while *L. kaempferi* enhanced its ability to absorb N-NH<sub>4</sub><sup>+</sup> under interspecific  
4 competition in mixed forest soil. Competition or facilitation modified the  
5 photosynthetic traits and nitrogen metabolism depending on the type of soil.  
6 Differences in these physiological processes contribute to divergent performance  
7 among individuals growing under interspecific or intraspecific competition, or in  
8 isolation.

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10 **Keywords:** C and N coordination, forest plantation, plant-plant interactions, plant-soil  
11 feedback.

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## 1. Introduction

Competition and facilitation are plant-plant interactions that are strongly influenced by resources. Facilitation indicates the positive impact of one plant on another through improving its recruitment, growth and survival (Sthultz et al., 2007). The magnitude or direction of net plant-plant interactions are largely determined by the absorption or exploration of soil resources (Boyden et al., 2005; Trinder et al., 2012; García-Cervigón et al., 2013; Loranger et al., 2017; Song et al., 2017), which are heterogeneously distributed in space. Soil properties (physical, chemical and microbial) also vary greatly among different environments. Changes or shifts between competition and facilitation among plants have been previously examined across abiotic stress gradients, but some of the results are contradictory (Bertness and Callaway, 1994; Boyden et al., 2005; Maestre et al., 2005; Sthultz et al., 2007; Biswas and Wagner, 2014; Guo et al., 2017). Most studies have been based on survival, growth rate, biomass production or nutrient absorption. It is still largely unknown, how competition or facilitation affects physiological characteristics related to photosynthesis and nitrogen metabolism in different soil conditions.

Niche complementarity has been proposed as an explanation for species coexistence and interactions. Interspecific differences in plant traits related to physiology (e.g. leaf nitrogen metabolism and photosynthesis) or morphology (e.g. root architecture) are often the basis to select species to reduce competition intensity or to sufficiently make

1 use of soil resources when constructing mixed-species plantations (reviewed by  
2 Richards et al., 2010). Cai et al. (2009) have revealed that in certain types of forests  
3 lianas fix more carbon and are more efficient in using water and nitrogen than trees,  
4 thus being better competitors (Schnitzer, 2005). However, few studies have examined  
5 whether tree species with a higher photosynthetic ability (carbon fixation) and  
6 nutrient efficiency (such as nitrogen) are expected to have superior competitiveness,  
7 especially when variation or shift between competition and facilitation are affected by  
8 differences in soil conditions.

9

10 Mixed-species plantations are known to have higher productivity and C sequestration  
11 than monocultures (Forrester et al., 2006; Kelty, 2006; Richards et al., 2010).  
12 Monoculture forest plantations always confront decreasing productivity during  
13 successive rotations, mainly due to a decline in soil qualities, including soil  
14 compaction, nutrient deficiencies or intraspecific allelopathy (reviewed by, e.g., Fox,  
15 2000; O’Hehir and Nambiar, 2010, Xia et al., 2016). Partitioning in the uptake of  
16 different nutrition forms is one factor regulating the results of interactions  
17 (Ahmad-Ramli et al., 2013; Chen et al., 2014). Chen et al. (2014) have found that  
18 *Populus cathayana* females have a strong ability to absorb  $\text{NH}_4^+$  and  $\text{NO}_3^-$  leading to  
19 a higher photosynthetic capacity and superior competitiveness compared to *Populus*  
20 males during competition under well-watered conditions. Absorbed  $\text{NO}_3^-$  can be  
21 converted into  $\text{NH}_4^+$  by series of enzymes, including nitrate reductase (NR). The  
22 converted  $\text{NH}_4^+$  and the absorbed  $\text{NH}_4^+$  can be further converted into glutamine (Gln)

1 and glutamate (Glu) by glutamine synthetase (GS), glutamate synthase and glutamate  
2 dehydrogenase (GDH). Gln and Glu provide precursors for the biosynthesis of  
3 N-containing compounds, such as most amino acids (Forde and Lea, 2007) and  
4 photosynthetic-related compounds, particularly Rubisco and light-harvesting  
5 complexes (Zhu et al., 2008).

6

7 Larches are deciduous trees within the genus *Larix* with high economic and ecological  
8 value and they have a wide range of distribution across the Northern Hemisphere,  
9 including Siberia, northeastern China and Japan (Agathokleous et al., 2017). *Larix*  
10 *kaempferi* and *L. olgensis* are two important larch plantation species. Li et al. (2016)  
11 found that *L. kaempferi* has higher growth rates, net photosynthetic rates and total leaf  
12 non-structural carbohydrate contents, but a lower leaf  $\text{NO}_3^-$  concentration compared to  
13 *L. olgensis* in the absence of plant-plant interactions. We conducted two experiments  
14 to investigate the effects of intra- and interspecific interactions between *L. kaempferi*  
15 and *L. olgensis* under different soil conditions. Our previous results have suggested  
16 that the productivity of the two *Larix* species grown in mixture is greatly promoted  
17 under N fertilization (Guo et al., 2017). Most importantly, competition or facilitation  
18 of the two species responding to soil type and N fertilization showed great changes  
19 during the experimental period. Although significant increases in the contents of N  
20 and non-structural carbohydrates (NSC) were found under facilitation, there is still a  
21 need to further explore how competition or facilitation affect photosynthetic traits and  
22 nitrogen metabolism in different soil conditions. Interspecies differences in

1 physiology that result from plant-plant interactions compared to monoculture may be  
2 specific to soil conditions. Our main hypothesis was that growth rates, photosynthetic  
3 traits and nitrogen metabolism of one species are facilitated or inhibited by another  
4 depending on the soil conditions.

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## 2. Methods and materials

Two experiments were conducted from late October 2013 to early September 2015. In Experiment 1, soils from a *L. kaempferi* plantation and from a natural secondary forest were selected. In this experiment, we focused on the performance of *L. kaempferi*: is the interspecific relationship of *L. kaempferi* with *L. olgensis* different when using *L. kaempferi* soil or soil from a secondary natural forest? In Experiment 2, N fertilization was applied to the larch soil. In this experiment, we studied the effects of inter- and intraspecific interactions on growth and ecophysiological responses under N fertilization in *L. kaempferi* soil.

### 2.1. Study region and experimental design

The study was conducted at the Qingyuan Experimental Station of the Forest Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Liaoning Province, Northeast China (41°51'N, 124°54'E). The mean annual rainfall is 811 mm (80% in summer). The mean annual air temperature varies between 3.9 °C and 5.4 °C (the coldest month of January averaging -12.1 °C and the warmest month of July averaging 21.0 °C) (Lu et al., 2018). The two selected types of soil were from a *c.* twenty years old *L. kaempferi* plantation and from a natural secondary forest, where *Quercus mongolica* and *Juglans mandshurica* are the dominant tree species. Hereafter, we call the two soils larch soil and mixed forest soil, respectively. The properties of the larch

soil were as follows: pH 5.65, C 18.61 g kg<sup>-1</sup>, N 1.82 g kg<sup>-1</sup>, while those of the mixed forest soil were as follows: pH 6.24, C 42.78 g kg<sup>-1</sup>, N 3.89 g kg<sup>-1</sup>. One-year old seedlings of the two species with approximately the same crown size and height were selected from a local nursery garden to be used as experimental materials.

## 2.2. Experiment 1: soil type

The intra- and interspecific plant-plant interactions were designed as follows: monoculture *L. kaempferi* + *L. kaempferi* and *L. olgensis* + *L. olgensis* representing intraspecific interactions, and mixed cultures *L. kaempferi* + *L. olgensis* representing interspecific interactions. In late October, 2013, two seedlings were planted in each plastic pot (external diameter and height 56 cm and 33 cm, respectively). The layout of Experiment 1 was two (larch soil and mixed forest soil) × three (*L. kaempferi* + *L. kaempferi*, *L. olgensis* + *L. olgensis*, and *L. kaempferi* + *L. olgensis*).

## 2.3. Experiment 2: N fertilization

The Experiment 2 was performed only in the larch soil at the same time. Its layout was two (with and without N fertilization) × three (*L. kaempferi* + *L. kaempferi*, *L. olgensis* + *L. olgensis*, and *L. kaempferi* + *L. olgensis*). Urea (46.3% N) was applied as N fertilization and added twice a year, in May and June (5.1 g each time) during years 2014 and 2015. More detailed information is available in Guo et al. (2017).

1  
2 In addition, three individuals of each species were planted in isolation in each type of  
3 soil and in the fertilized soil at the same time. Based on our pervious results, *L.*  
4 *kaempferi* confronts strong competition from *L. olgensis* in the larch soil without N  
5 fertilization (in contrary, *L. olgensis* is facilitated by *L. kaempferi*), whereas it is  
6 facilitated significantly by *L. olgensis* in the mixed forest soil (in contrary, *L. olgensis*  
7 confronts strong competition from *L. kaempferi*. Guo et al. 2017).

#### 8 9 2.4. Sampling and measurements

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11 The final height and stem diameter of five replicates from each treatment were  
12 measured to calculate the growth rates in late August, 2015. The relative height and  
13 stem diameter growth rates were calculated as follows: relative growth rate =  $(\ln$   
14  $(\text{final}) - \ln(\text{initial})) / (t_2 - t_1)$ , where the denominator is the time elapsed between the  
15 initial and final measurements.

16  
17 The net photosynthetic rates (*Pn*) were measured by using a portable photosynthesis  
18 system (LI-6400; Li-Cor Inc., Lincoln, NE, USA) at the end of July and beginning of  
19 September, 2015. Healthy leaves were selected. From each treatment, four replicates  
20 were chosen randomly. The measurements were performed between 08:30 and 11:30  
21 h under the following conditions: leaf temperature 25 °C, relative air humidity from  
22 69% to 71%, photosynthetic photon flux density 1500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  and ambient CO<sub>2</sub>

1 concentration  $350 \pm 5 \mu\text{mol mol}^{-1}$ . After that, some leaves were immediately sampled  
2 and dried at  $70^\circ\text{C}$  for 72 hours. The dried leaves were ground into powder, and leaf N  
3 concentration was determined by the semi-micro Kjeldahl method (Mitchell, 1998).  
4 Nitrogen concentration variation from July to September was calculated using the  
5 following formula:  $\text{N concentration variation} = [\text{N concentration}_{\text{Sep}} - \text{N}$   
6  $\text{concentration}_{\text{Jul}}] \times 100\% / \text{N concentration}_{\text{Jul}}$ , where  $\text{N concentration}_{\text{Jul}}$  and  $\text{N}$   
7  $\text{concentration}_{\text{Sep}}$  represent leaf N concentration in late July and early September,  
8 respectively. Higher concentration variation indicates greater N absorption during this  
9 period.

10

11 Fresh leaves were sampled after  $P_n$  measurements in early September, 2015. For the  
12 measurement of chlorophyll pigments conducted according to the method of  
13 Lichtenthaler (1987), four replicates from each treatment were chosen. The total  
14 chlorophyll content ( $Tchl$ ) was the sum of chlorophyll  $a$  and  $b$ . Three or four  
15 replicates of fresh leaves from each treatment were sampled to determine the activities  
16 of GS, GDH and NR by using ELISA kits (Shanghai Enzyme-linked Biotechnology  
17 Co., Ltd.).

18

19 Healthy leaves (1-2 mm in length) were used for transmission electron microscopy  
20 (TEM) observations by H-600IV TEM (Hitachi, Tokyo, Japan) in early September,  
21 2015. The specific procedures followed the descriptions of Chen et al. (2014).

22

1  $\text{NH}_4^{15}\text{NO}_3$  and  $^{15}\text{NH}_4\text{NO}_3$  were used to reveal nitrogen uptake in different types of  
2 soil. Three randomly chosen cuttings per treatment were supplied either with 60 mg  
3  $\text{NH}_4^{15}\text{NO}_3$  or 60 mg  $^{15}\text{NH}_4\text{NO}_3$  per pot. Then, 72 h later, leaves from the upper parts  
4 of individuals were sampled from each treatment and dried at 70 °C for 72 hours. The  
5 ground powder was used for the analysis of the  $^{15}\text{N}$  isotope composition ( $\delta^{15}\text{N}$ ) by  
6 Isotope Ratio Mass Spectrometer (DELTA V Advantage, Thermo Fisher Scientific,  
7 Inc., Waltham, Massachusetts, USA). In addition, fine powder of leaf samples (six  
8 replicates from each treatment) was used to determine the carbon isotope composition  
9 ( $\delta^{13}\text{C}$ ). The  $^{13}\text{C}/^{12}\text{C}$  ratio was determined by Isotope Ratio Mass Spectrometer  
10 (DELTA V Advantage, Thermo Fisher Scientific, Inc., Waltham, Massachusetts,  
11 USA).

12

13 After all abovementioned measurements, individuals were harvested on the 4<sup>th</sup> of  
14 September, 2015. All harvested materials were dried at 70 °C for 72 hours. The dried  
15 leaves were ground into powder to determine non-structural carbohydrates (starch and  
16 soluble sugar) and hydrolysable amino acids of leaves. Total non-structural  
17 carbohydrates (TNC) were defined as the sum of starch and soluble sugars. The  
18 methods used to determine non-structural carbohydrates and amino acids followed  
19 those of Guo et al. (2017) and Chen et al. (2015), respectively.

20

## 21 *2.5. Data analysis*

22

1 Data were checked for normality and homogeneity of variances, and values were  
2 log-transformed when needed. Three-way analyses of variance (ANOVA) to  
3 determine the effects of factors in Experiment 1 and Experiment 2, respectively. After  
4 a significant interactive effect of factors was found, Tukey's *b* tests were conducted as  
5 post hoc tests to discover differences among treatments. All data were analyzed with  
6 the software Statistical Package for the Social Science (SPSS) version 20.0.

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### 21 **3. Results**

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### 3.1. Growth rates

The soil type, N fertilization and interactions significantly affected the growth rates of both species (Fig. 1). The larch soil showed a negative effect on the growth of *L. kaempferi*, particularly on the relative growth of stem diameter, compared to the effect of the mixed forest soil. N fertilization removed the negative effect and promoted the growth of *L. kaempferi* as well as that of *L. olgensis*. *L. olgensis* facilitated the stem growth of *L. kaempferi* in both mixed forest soil and in larch soil with N fertilization (larch soil N+; Fig. 1a).

### 3.2. Photosynthetic traits

The soil type (So) alone had no or only a marginally significant effect on *Chl a*, *Chl b*, *Caro*, *Thcl* and *Chl a/Chl b*. However, significant mutual effects between soil type and interactions (Int) were observed (Table 1). When the trees were grown in mixture, the *Chl a/Chl b* ratio of *L. kaempferi* was significantly higher in mixed forest soil than in larch soil without N fertilization (larch N- soil). N fertilization promoted the concentrations of *Chl a*, *Chl b* and *Thcl* in both species. In *L. kaempferi*, the *Chl a/Chl b* ratio was lower when grown in mixture compared to monoculture in larch soil without fertilization. In *L. olgensis*, the *Chl a/Chl b* ratio was significantly higher when grown in mixture compared to monoculture under N fertilization in larch soil. The soil type affected *Pn* and PNUE in late July and early September, 2015 (Fig.

2a-d). Under interspecific interactions in mixed forest soil, *L. kaempferi* exhibited a stronger net photosynthetic rate ( $P_n$ ), while  $P_n$  and PNUE of *L. olgensis* declined (Fig. 2a, b).

The factor Int showed a significant effect on the non-structural carbohydrate concentration of leaves (Fig. 3). In both species, there was a tendency that leaf starch and TNC were higher under interspecific interactions than in monoculture in both larch N- soil and in mixed forest soil (Fig. 3a, c). Leaf TNC of *L. olgensis* was significantly higher under interspecific interactions than when grown in monoculture in the larch N+ soil (Fig. 3c).

Observations on the mesophyll cells showed that the two species possessed differences in chloroplasts (Fig. 4). The granum sizes of *L. kaempferi* chloroplasts were bigger and the granum numbers were greater than in *L. olgensis* in the same treatment. Plant-plant interactions and soil type affected the number of chloroplasts, especially in *L. kaempferi*. Its chloroplast numbers tended to be lower in mixture than in monoculture in larch N- soil (Fig. 4a, b). However, in mixed forest soil, the chloroplast number of *L. kaempferi* was greater in a mixture than in monoculture (Fig. 4i, j). The  $\delta^{13}\text{C}$  value was affected only by the mutual effect between the plant-plant interaction and soil type (Fig. 5a).

### 3.3. N metabolism



1

2  $\text{NO}_3^-$  was the main N form absorbed by the two species. In larch N- soil, *L. olgensis*  
3 enhanced its ability to absorb N- $\text{NO}_3^-$  in mixture (Fig. 5b), while in mixed forest soil,  
4 *L. kaempferi* enhanced its ability to absorb N- $\text{NH}_4^+$  in mixture (Fig. 5c). Nitrogen  
5 fertilization influenced the leaf N concentration in both July and early September (Fig.  
6 6a, b). Plant-plant interactions affected the leaf N concentration only early September  
7 when the N concentration tended to be higher in mixture than in monoculture in both  
8 species (Fig. 6b). Generally, the N concentration variation from late July to early  
9 September tended to be higher when the two *Larix* were grown in mixture (Fig. 6c).  
10 The activities of GDH and GS were not significantly affected by either factor (Fig. 7a,  
11 b). The activity of NR was significantly affected only by the mutual effect between  
12 species and plant-plant interactions (Fig. 7c). Our results indicated that none of the  
13 hydrolysable amino acids showed significant differences (Table 2). However,  
14 according to the results of the three-way ANOVA, the mutual effect between the soil  
15 type and plant-plant interactions marginally affected the concentrations of some  
16 amino acids, such as aspartate, glycine and cysteine (see supplementary Table S1).  
17 Concentrations of most amino acids were a little higher under interspecific  
18 interactions in both species compared with those under monoculture in the larch N-  
19 soil, whereas this tendency was opposite in mixed forest soil (Table 2).

20

#### 21 **4. Discussion**

22

1 In our study, we found that photosynthetic traits and nitrogen metabolism characters  
2 were affected by competition or facilitation in different soil conditions.

#### 3 4 *4.1. Negative effects of larch soil*

5  
6 A given plant species can alter soil environment in a way that decreases its own  
7 growth rate relative to that of others, resulting in a negative feedback (Harrison and  
8 Bardgett, 2010; Xia et al., 2016). Plants release a wide range of molecules that play  
9 important roles in interactions between roots and soil organisms, such as amino acids,  
10 sugars and proteins (Haichar et al., 2014; Zhang et al., 2015). Our results indicated  
11 that the growth rates of *L. kaempferi* were negatively influenced by *L. kaempferi* soil  
12 without N fertilization (larch N- soil). A negative plant-soil feedback caused by  
13 changes in nutrient availability and microbial communities (Harrison and Bardgett,  
14 2010) leads to changes in interspecific competition (Hendriks et al., 2015).  
15 Differences in chemical properties are one reason causing differences on the leaf level  
16 between the two types of soil (Miatto et al., 2016). In this study, the soil type was an  
17 important factor to drive differences in photosynthesis-related traits and in some  
18 nitrogen metabolism processes. For example, the effects of soil type on *Pn* and PNUE  
19 were remarkable in late July and early September. We found that the soil type  
20 influenced the uptake of  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N, especially under the mutual effect with  
21 plant-plant interactions. The use of N fertilization is suggested to be an efficient forest  
22 management approach to solve the problem of declining productivity along successive

1 rotations of plantations (Fox, 2000; O’Hehir and Nambiar, 2010). In our study,  
2 nitrogen fertilization removed the negative effect, accelerated growth in *L. kaempferi*  
3 and promoted biomass production (Guo et al., 2017), by not only increasing soil N  
4 availability but also by altering microbial communities (Zechmeister-Boltenstern et al.,  
5 2011). We have found that nitrogen fertilization altered alpha diversity and  
6 composition of bacteria and fungi in the rhizosphere soil of the two species (data  
7 unpublished).

8

#### 9 4.2. Effects of competition or facilitation

10

11 Previously, isolated *L. kaempferi* was thought to have superior physiological leaf  
12 nutrition traits, because it displayed higher *Pn*, PNUE and photosynthetic N use  
13 efficiency compared to isolated *L. olgensis* under N and P fertilization (Li et al., 2016).  
14 However, our study did not show such clear species differences, mainly due to the  
15 effects of intra- or interspecific interactions. Plants have to optimize performance in  
16 morphology and physiological activity when they share resources with their  
17 conspecific or heterospecific neighbors (Kozovits et al., 2005; Cai et al., 2009; Duan  
18 et al., 2014). Our results indicated that *Pn* of *L. kaempferi* was facilitated while that of  
19 *L. olgensis* was inhibited by interspecific interaction in late July in mixed forest soil.  
20 The effects of competition and facilitation on *Pn*, and PNUE are evidently complex  
21 dynamic processes. It has been shown that higher *Pn* (mass-based), PNUE and  $\delta^{13}\text{C}$   
22 values (higher  $\delta^{13}\text{C}$  indicating higher integrated water-use efficiency) in lianas than in

1 trees throughout the year (especially during the drought season) confers a competitive  
2 advantage to lianas during the dry season and suggests that lianas capture resources  
3 more efficiently than trees (Cai et al., 2009).

4

5 The mass-based photosynthetic rate reflects the influence of the leaf structure on CO<sub>2</sub>  
6 uptake (Lichtenthaler et al., 2007). The proportion of cell wall constituents range  
7 between 18-70% of leaf dry mass and is typically associated with the fraction of leaf  
8 N invested in photosynthetic proteins (Onoda et al., 2017). Cell walls (CW) of *L.*  
9 *kaempferi* were thicker under N fertilization and affected by competition or  
10 facilitation, which may lead to differences in leaf morphology (e.g. increasing leaf  
11 weight).

12

13 Leaf chlorophyll pigments that are directly related to photosynthesis provide valuable  
14 information about the physiological status of plants (Lichtenthaler et al., 1981, 1982,  
15 2007). The organization of the chlorophyll pigments and the relative levels of *Chl b*  
16 and *Tchl* as well as the ratio of *Chl a* to *Chl b* showed considerable differences among  
17 treatments. *Chl a/b* variation may be positively related with the number of  
18 chloroplasts in the mesophyll structures of leaves. For example, *Chl a/b* of *L.*  
19 *kaempferi* under interspecific interactions was significantly lower than that under  
20 monoculture in the larch N- soil. Leaves with higher *Chl a/b* seemed to have more  
21 chloroplasts. Significantly higher values of *Chl a/b* were found in sun leaves and in  
22 the sunlit leaves from the upper canopy level, characterized by a higher

1 light-saturation of photosynthesis and higher chloroplast numbers (see Lichtenthaler  
2 et al. 1981) compared to shade leaves and leaves from the lower canopy level  
3 (Lichtenthaler et al., 1981, 2007; Hölscher, 2004). In addition, higher *Chl a/b*  
4 indicated the presence of fewer light-harvesting chlorophyll proteins (LHCII) and  
5 probably a larger number of reaction center pigment proteins (Lichtenthaler et al.,  
6 1981, 1982).

7  
8 Non-structural carbohydrates play a key role in physiological processes and are  
9 thought to reflect a balance between carbon supply (photosynthesis) and demand  
10 (such as growth and respiration) (Koch, 2004; Sala et al., 2012). Liu et al. (2004) have  
11 demonstrated that the total carbohydrate content (sugars and starch) of European  
12 beech (*Fagus sylvatica*) declined when it confronted strong competition with Norway  
13 spruce (*Picea abies*) when exposed to elevated CO<sub>2</sub>. Species was the primary factor to  
14 affect leaf starch concentrations, being higher in *L. kaempferi* than in *L. olgensis*. The  
15 result was consistent with the leaf mesophyll structure: the number and size of starch  
16 grains were greater in *L. kaempferi* compared to *L. olgensis*. It implied that the two  
17 larch species may differ in starch synthesis within a chloroplast or in the transport of  
18 triose phosphate from a chloroplast to cytoplasm. Interspecific interactions greatly  
19 affected the non-structural carbohydrates of leaves, particularly in *L. olgensis*, which  
20 had higher leaf TNC when grown in mixture. However, the mechanism of how  
21 competition or facilitation modulates leaf TNC remains unresolved, because we did  
22 not accurately measure how much carbon was fixed and exported for growth,

1 respiration, defense and secretion.

2

3 The partitioning in nutrient uptake regulates competitive relationships (Ahmad-Ramli  
4 et al., 2013; Chen et al., 2014). We provided evidence that the two larch species  
5 grown together show slight differences in N-source absorption partitioning. Most  
6  $\text{NH}_4^+$  can be assimilated locally, while the rest is transported to leaves or other parts,  
7 but most absorbed  $\text{NO}_3^-$  is transported to leaves, where it is assimilated by a series of  
8 enzymes after uptake into roots (Black et al., 2002; Li et al., 2012; Xu et al., 2012).  
9 Luo et al. (2013) found that the activities of leaf NR and GS show no differences  
10 between fast and slowly growing species in response to different N availabilities.  
11 However, the uptake of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , and transcript levels of most ammonium  
12 (AMTs) and nitrate (NRTs) transporter genes in leaves showed considerable changes.  
13 The isolated *L. kaempferi* has a higher growth rate than isolated *L. olgensis* (Yin et al.,  
14 2008; Li et al., 2016), while the growth rates of the two species also show differences  
15 caused by plant-plant interactions, as discovered in the experiments of the present  
16 study. However, the activities of leaf GDH, GS and NR showed no or only slight  
17 variation between different soil conditions.

18

19 Amino acids are used for protein biosynthesis and N storage (e.g. arginine and  
20 arginine-rich proteins). In our study, the concentrations of N and amino acids showed  
21 no significant changes among treatments. The probable reason was the dilution effect  
22 of biomass. Competitive responses are dynamic processes that change along with

1 nitrogen capture and biomass production (Trinder et al., 2012). Variation in leaf N  
2 concentrations tended to be higher when the two species were grown together  
3 compared to monoculture, which reflects their differences in the preferred N form or  
4 ability to absorb or store N resources when interacting with heterospecific and  
5 conspecific plants.

6

#### 7 *4.3. Carbon and nitrogen interactions*

8

9 Plants must integrate external and internal signals to modify their growth. It has been  
10 reported that starch integrates the metabolic status and the total protein content, thus  
11 suggesting that the regulatory network that determines starch and protein contents  
12 contributes to the regulation of biomass production (Matt et al., 2001; Sulpice et al.,  
13 2009). Diverse conclusions have been made in studies involving plants grown in  
14 monoculture or mixture, for example, by Liu et al. (2004) and Kozovits et al. (2005).  
15 Knowledge about the performance of plants acquired from plants growing in  
16 monoculture (or in isolation) may not be transferred to plants grown under  
17 interspecific interactions (Kozovits et al., 2005). Our results on the growth and  
18 physiological traits of the two studied species growing under interaction conditions  
19 differed from those by Li et al. (2016), where experimental individuals were planted  
20 in isolation. This implies that the coordination or balance between carbon and  
21 nitrogen was influenced by competition or facilitation, which enabled interacting  
22 plants to optimize their competitive performance.

1

## 2 **5. Conclusions**

3

4 Plants integrate external and internal signals to regulate the balance of carbon and  
5 nitrogen, and to optimize their performance in different conditions. Our findings  
6 indicated that physiological processes are affected by competition or facilitation under  
7 different types of soil. Divergent responses and performance under intra- and  
8 interspecific interactions in varied conditions reflect plant adaptation.

9

10 **Author Contribution Statement** Qingxue Guo had the main responsibility for data  
11 collection, analysis and writing, Haifeng Song and Jieyu Kang had a significant  
12 contribution to data collection and analysis, Helena Korpelainen had a significant  
13 contribution to the interpretation of data and manuscript preparation, and Chunyang  
14 Li (the corresponding author) had the overall responsibility for experimental design  
15 and project management.

16

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19

20 **Conflict of interest** The authors declare that they have no conflicts of interest.

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22



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1 **Table 1** Leaf pigments of the two *Larix* species in early September, 2015. *Chl a*: chlorophyll *a*, *Chl b*: chlorophyll *b*, *Caro*: carotenoid, *Tchl*: chl *a* + chl *b*.

Species	Treatment	<i>Chl a</i> (mg·g <sup>-1</sup> FW)		<i>Chl b</i> (mg·g <sup>-1</sup> FW)		<i>Caro</i> (mg·g <sup>-1</sup> FW)		<i>Tchl</i> (mg·g <sup>-1</sup> FW)		<i>Chl a</i> : <i>Chl b</i>	
<i>L. kaempferi</i>	Inter	0.64 ± 0.05 <b>AB</b>		0.15 ± 0.01cd <b>BC</b>		0.03 ± 0.00ab <b>AB</b>		0.79 ± 0.07bc <b>ABC</b>		4.34 ± 0.07bc <b>C</b>	
		<b>0.66 ± 0.04AB</b>		<b>0.10 ± 0.01C</b>		<b>0.03 ± 0.00AB</b>		<b>0.75 ± 0.05BC</b>		<b>6.94 ± 0.34A</b>	
	Intra	0.50 ± 0.01 <b>B</b>		0.09 ± 0.01e <b>C</b>		0.03 ± 0.00b <b>B</b>		0.59 ± 0.02d <b>C</b>		5.83 ± 0.39a <b>AB</b>	
		<b>0.60(0.03)B</b>		<b>0.13 ± 0.01BC</b>		<b>0.03 ± 0.00AB</b>		<b>0.73 ± 0.05C</b>		<b>4.51 ± 0.26BC</b>	
	Inter-N	0.76 ± 0.04		0.18± 0.01bc		0.04 ± 0.01ab		0.94 ± 0.05ab		4.20 ± 0.13bc	
	Intra-N	0.67 ± 0.03		0.15 ± 0.00cd		0.04 ± 0.00ab		0.81 ± 0.03bc		4.57 ± 0.17bc	
<i>L. olgensis</i>	Inter	0.77 ± 0.02 <b>A</b>		0.20 ± 0.01b <b>A</b>		0.04 ± 0.01ab <b>A</b>		0.97 ± 0.02ab <b>A</b>		3.83 ± 0.22cd <b>C</b>	
		<b>0.65(0.05)AB</b>		<b>0.14 ± 0.02BC</b>		<b>0.03 ± 0.00AB</b>		<b>0.79 ± 0.07ABC</b>		<b>5.03 ± 0.47BC</b>	
	Intra	0.61 ± 0.04 <b>AB</b>		0.12 ± 0.01de <b>BC</b>		0.03 ± 0.00ab <b>AB</b>		0.73 ± 0.05c <b>C</b>		5.08 ± 0.32ab <b>BC</b>	
		<b>0.78(0.02)A</b>		<b>0.17 ± 0.02AB</b>		<b>0.05 ± 0.00A</b>		<b>0.95 ± 0.03AB</b>		<b>4.65 ± 0.35BC</b>	
	Inter-N	0.80 ± 0.02		0.17 ± 0.00bc		0.04 ± 0.00ab		0.98 ± 0.01ab		4.66 ± 0.20bc	
	Intra-N	0.81 ± 0.08		0.28 ± 0.01a		0.05 ± 0.00a		1.09 ± 0.08a		2.95 ± 0.31d	
	<i>P</i> : <i>F</i> <sub>S</sub>	0.001	<b>0.001</b>	0.000	<b>0.000</b>	0.039	<b>0.018</b>	0.000	<b>0.000</b>	0.002	<b>0.003</b>
	<i>P</i> : <i>F</i> <sub>Int</sub>	0.004	<b>0.041</b>	0.016	<b>0.091</b>	0.105	<b>0.440</b>	0.001	<b>0.040</b>	0.054	<b>0.937</b>
	<i>P</i> : <i>F</i> <sub>N (So)</sub>	0.000	<b>0.147</b>	0.000	<b>0.542</b>	0.009	<b>0.227</b>	0.000	<b>0.329</b>	0.001	<b>0.033</b>
	<i>P</i> : <i>F</i> <sub>S×Int</sub>	0.518	<b>0.147</b>	0.000	<b>0.491</b>	0.387	<b>0.327</b>	0.144	<b>0.341</b>	0.003	<b>0.057</b>
	<i>P</i> : <i>F</i> <sub>S×N (×So)</sub>	0.693	<b>0.584</b>	0.208	<b>0.770</b>	0.750	<b>0.927</b>	0.526	<b>0.612</b>	0.897	<b>0.582</b>
	<i>P</i> : <i>F</i> <sub>Int×N (×So)</sub>	0.078	<b>0.002</b>	0.000	<b>0.000</b>	0.030	<b>0.002</b>	0.002	<b>0.000</b>	0.000	<b>0.000</b>
	<i>P</i> : <i>F</i> <sub>S×Int×N (×So)</sub>	0.301	<b>0.063</b>	0.000	<b>0.633</b>	0.238	<b>0.191</b>	0.102	<b>0.112</b>	0.014	<b>0.018</b>

2 Inter, interspecific interaction; Intra, intraspecific interaction; Inter-N, interspecific interaction under N fertilization; Intra-N, intraspecific interaction under N fertilization. *F<sub>S</sub>*,  
3 species effect; *F<sub>Int</sub>*, plant-plant interaction effect; *F<sub>N(So)</sub>*, N fertilization or soil type effect; *F<sub>S×Int</sub>*, species × plant-plant interaction effect; *F<sub>S×N(×So)</sub>*, N fertilization or soil type ×  
4 species effect; *F<sub>Int×N(×So)</sub>*, N fertilization or soil type × plant-plant interaction effect; *F<sub>S×Int×N(×So)</sub>*, N fertilization or soil type × species × plant-plant interaction. In Experiment 1  
5 (two soil types), different capital letters in the same column indicate significant differences at *P* < 0.05 based on Tukey's *b* (values in bold). In Experiment 2 (N fertilization),  
6 values followed by different lowercase letters in the same column are significantly different at *P* < 0.05 based on Tukey's *b* analysis.

1 **Table 2** Amino acids concentrations of the two *Larix* species in each treatment in early September, 2015.

Amino acid (g·100g <sup>-1</sup> DW)	<i>L. kaempferi</i>				<i>L. olgensis</i>			
	Inter	Intra	Inter-N	Intra-N	Inter	Intra	Inter-N	Intra-N
Aspartate	1.14 ± 0.08A <b>1.07 ± 0.10A</b>	0.99 ± 0.08A <b>1.21 ± 0.06A</b>	1.07 ± 0.11	1.11 ± 0.12	1.22 ± 0.10A <b>1.09 ± 0.04A</b>	1.11 ± 0.00A <b>1.21 ± 0.08A</b>	1.05 ± 0.13	1.11 ± 0.06
Threonine	0.58 ± 0.04 <b>0.55 ± 0.04</b>	0.49 ± 0.04 <b>0.57 ± 0.03</b>	0.55 ± 0.05	0.57 ± 0.06	0.61 ± 0.05 <b>0.58 ± 0.03</b>	0.57 ± 0.00 <b>0.60 ± 0.02</b>	0.54 ± 0.07	0.56 ± 0.03
Serine	0.55 ± 0.04 <b>0.48 ± 0.04</b>	0.46 ± 0.03 <b>0.51 ± 0.02</b>	0.51 ± 0.05	0.53 ± 0.06	0.55 ± 0.05 <b>0.51 ± 0.02</b>	0.53 ± 0.00 <b>0.54 ± 0.03</b>	0.50 ± 0.06	0.52 ± 0.03
Glutamate	1.29 ± 0.10 <b>1.20 ± 0.08</b>	1.09 ± 0.08 <b>1.28 ± 0.06</b>	1.20 ± 0.12	1.26 ± 0.14	1.33 ± 0.11 <b>1.31 ± 0.05</b>	1.32 ± 0.03 <b>1.29 ± 0.05</b>	1.21 ± 0.15	1.25 ± 0.07
Glycine	0.69 ± 0.06A <b>0.65 ± 0.06A</b>	0.60 ± 0.05A <b>0.73 ± 0.03A</b>	0.64 ± 0.06	0.67 ± 0.08	0.74 ± 0.06A <b>0.68 ± 0.03 A</b>	0.66 ± 0.01A <b>0.72 ± 0.05A</b>	0.64 ± 0.08	0.66 ± 0.04
Alanine	0.79 ± 0.06 <b>0.75 ± 0.06</b>	0.66 ± 0.05 <b>0.78 ± 0.04</b>	0.73 ± 0.07	0.76 ± 0.09	0.81 ± 0.07 <b>0.82 ± 0.04</b>	0.80 ± 0.02 <b>0.80 ± 0.03</b>	0.73 ± 0.09	0.75 ± 0.04
Cysteine	0.05 ± 0.01aA <b>0.03 ± 0.01A</b>	0.04 ± 0.01aA <b>0.04 ± 0.00A</b>	0.04 ± 0.01a	0.04 ± 0.01a	0.05 ± 0.01aA <b>0.03 ± 0.01A</b>	0.04 ± 0.00aA <b>0.04 ± 0.01A</b>	0.03 ± 0.00a	0.04 ± 0.00a
Valine	0.74 ± 0.06 <b>0.72 ± 0.05</b>	0.64 ± 0.05 <b>0.80 ± 0.04</b>	0.69 ± 0.07	0.73 ± 0.08	0.80 ± 0.06 <b>0.76 ± 0.02</b>	0.74 ± 0.01 <b>0.78 ± 0.04</b>	0.69 ± 0.09	0.72 ± 0.04
Methionine	0.17 ± 0.04A <b>0.07 ± 0.02A</b>	0.07 ± 0.01A <b>0.07 ± 0.00A</b>	0.10 ± 0.02	0.08 ± 0.01	0.10 ± 0.02A <b>0.05 ± 0.01A</b>	0.10 ± 0.04A <b>0.11 ± 0.04A</b>	0.06 ± 0.01	0.06 ± 0.00
Isoleucine	0.61 ± 0.05A <b>0.59 ± 0.06A</b>	0.53 ± 0.04A <b>0.65 ± 0.03A</b>	0.56 ± 0.05	0.59 ± 0.06	0.66 ± 0.05A <b>0.60 ± 0.02A</b>	0.58 ± 0.00A <b>0.63 ± 0.04A</b>	0.56 ± 0.07	0.57 ± 0.03
Leucine	1.16 ± 0.09 <b>1.09 ± 0.08</b>	0.98 ± 0.08 <b>1.14 ± 0.05</b>	1.08 ± 0.11	1.12 ± 0.13	1.22 ± 0.10 <b>1.19 ± 0.04</b>	1.16 ± 0.02 <b>1.16 ± 0.04</b>	1.07 ± 0.14	1.10 ± 0.06
Tyrosine	0.45 ± 0.04A	0.36 ± 0.04A	0.40 ± 0.05	0.42 ± 0.06	0.49 ± 0.05A	0.43 ± 0.01A	0.37 ± 0.04	0.40 ± 0.02

	<b>0.39 ± 0.03A</b>	<b>0.45 ± 0.02A</b>			<b>0.45 ± 0.02A</b>	<b>0.46 ± 0.03A</b>		
Phenylalanine	0.73 ± 0.06	0.62 ± 0.05	0.69 ± 0.07	0.70 ± 0.08	0.78 ± 0.07	0.72 ± 0.01	0.67 ± 0.08	0.70 ± 0.03
	<b>0.69 ± 0.05</b>	<b>0.74 ± 0.03</b>			<b>0.75 ± 0.03</b>	<b>0.74 ± 0.03</b>		
Lysine	0.57 ± 0.04A	0.55 ± 0.03A	0.56 ± 0.07	0.62 ± 0.07	0.65 ± 0.05A	0.58 ± 0.03A	0.58 ± 0.07	0.58 ± 0.05
	<b>0.62 ± 0.06A</b>	<b>0.71 ± 0.04A</b>			<b>0.58 ± 0.02A</b>	<b>0.58 ± 0.03A</b>		
NH <sub>4</sub> <sup>+</sup>	0.16 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.00	0.14 ± 0.01	0.16 ± 0.02
	<b>0.14 ± 0.01</b>	<b>0.16 ± 0.01</b>			<b>0.15 ± 0.00</b>	<b>0.15 ± 0.01</b>		
Histidine	0.29 ± 0.02	0.25 ± 0.02	0.27 ± 0.03	0.28 ± 0.03	0.30 ± 0.02	0.29 ± 0.00	0.27 ± 0.03	0.28 ± 0.02
	<b>0.29 ± 0.02</b>	<b>0.29 ± 0.01</b>			<b>0.31 ± 0.01</b>	<b>0.32 ± 0.02</b>		
Arginine	0.77 ± 0.07	0.64 ± 0.05	0.74 ± 0.08	0.82 ± 0.15	0.80 ± 0.07	0.77 ± 0.01	0.74 ± 0.10	0.81 ± 0.04
	<b>0.71 ± 0.05</b>	<b>0.75 ± 0.04</b>			<b>0.76 ± 0.03</b>	<b>0.81 ± 0.05</b>		
Proline	0.62 ± 0.04	0.53 ± 0.04	0.59 ± 0.06	0.60 ± 0.07	0.65 ± 0.05	0.67 ± 0.04	0.59 ± 0.07	0.58 ± 0.04
	<b>0.61 ± 0.06</b>	<b>0.58 ± 0.03</b>			<b>0.71 ± 0.06</b>	<b>0.57 ± 0.02</b>		
Total amino acid	11.36 ± 0.87	9.64 ± 0.74	10.56 ± 1.07	11.04 ± 1.28	11.93 ± 1.01	11.21 ± 0.12	10.42 ± 1.29	10.87 ± 0.57
	<b>10.67 ± 0.80</b>	<b>11.46 ± 0.54</b>			<b>11.33 ± 0.41</b>	<b>11.49 ± 0.51</b>		

- 1 Inter, interspecific interaction; Intra, intraspecific interaction; Inter-N, interspecific interaction under N fertilization; Intra-N, intraspecific interaction under N fertilization.
- 2 Tukey's *b* tests were conducted as post hoc tests to discover differences among treatments, when a significant factor interaction was observed after ANOVA analysis. Values
- 3 in bold indicate results among treatments between the two soil types (Experiment 1); same capital letters indicate no significant difference.

## Figure legends

**Figure 1** Relative growth rates as diameter (a) and stem height (b) in two *Larix* species. Inter and Intra refer to inter- and intraspecific interaction, respectively; Inter-N and Intra-N refer to inter- and intraspecific interaction under N fertilization, respectively. N: N fertilization effect, S: species effect; Int: plant-plant interaction effect, N×S: N fertilization × species effect, N×Int: N fertilization × plant-plant interaction effect, S×Int: species × plant-plant interaction effect, N×S×Int: N fertilization × species × plant-plant interaction effect, So: soil type effect, S×So: species × soil type effect, S×So×Int: species × soil type × plant-plant interaction. In Experiment 1 (two soil types), different capital letters indicate significant differences at  $P < 0.05$ . The red frame indicates treatments in the mixed forest soil. In Experiment 2 (N fertilization), values followed by different lowercase letters are significantly different at  $P < 0.05$ . Black and white bars indicate *L. kaempferi* and *L. olegensis*, respectively.

**Figure 2** Net photosynthetic rate  $P_n$  (a and c) and photosynthetic nitrogen use efficiency (PNUE, b and d) in two *Larix* species in late July and early September. Detailed information of symbols shown in **Fig. 1**.

**Figure 3** Non-structural carbohydrates of leaves including starch, soluble sugars and total non-structural carbohydrates (TNC) in two *Larix* species in early September, 2015. (a) Leaf starch concentration, (b) leaf soluble sugar concentration, (c) leaf TNC.

Detailed information of symbols shown in **Fig. 1**.

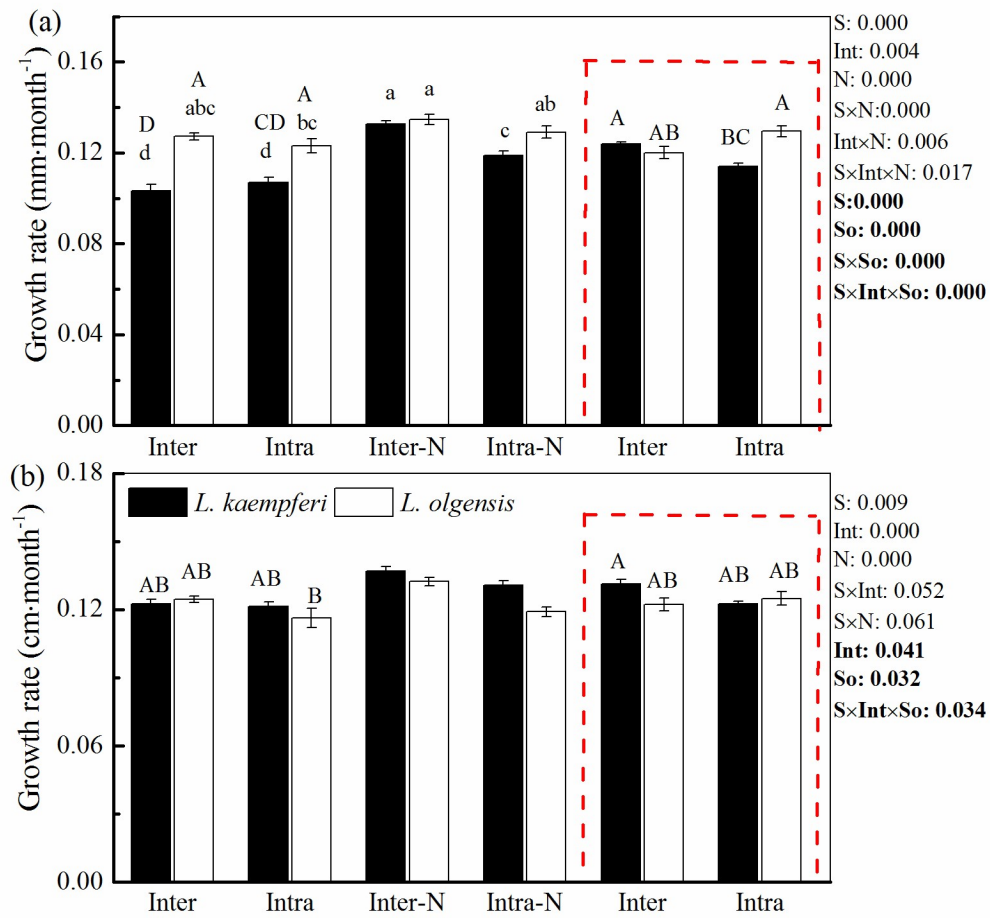
**Figure 4** Ultrastructure of mesophyll cells in two *Larix* species in early September, 2015. (a) - (d): *L. kaempferi* in larch soil, (a): Inter, (b): Intra, (c): Inter-N, (d): Intra-N; (e) - (h): *L. olegensis* in larch soil, (e): Inter, (f): Intra, (g): Inter-N, (h): Intra-N; (i) - (j): *L. kaempferi* in mixed forest soil, (i): Inter, (j): Intra, (k) - (l): *L. olegensis* in mixed forest soil, (k): Inter, (l): Intra. The bar indicates 1  $\mu\text{m}$  (c). CW: cell wall, Ch: chloroplast, SG: starch granum. Detailed information of symbols shown in **Fig. 1**.

**Figure 5**  $\delta^{13}\text{C}$  (a),  $^{15}\text{NO}_3^-$ -N (b) and  $^{15}\text{NH}_4^+$ -N (c) of two *Larix* species in early September, 2015. Detailed information of symbols shown in **Fig. 1**.

**Figure 6** Leaf nitrogen concentrations in two *Larix* species at the end of July (a) and in early September (b), and variation in leaf nitrogen concentration from late July to early September in 2015 (c). Calculations of nitrogen concentrations explained in the Methods and materials section. Detailed information of symbols shown in **Fig. 1**.

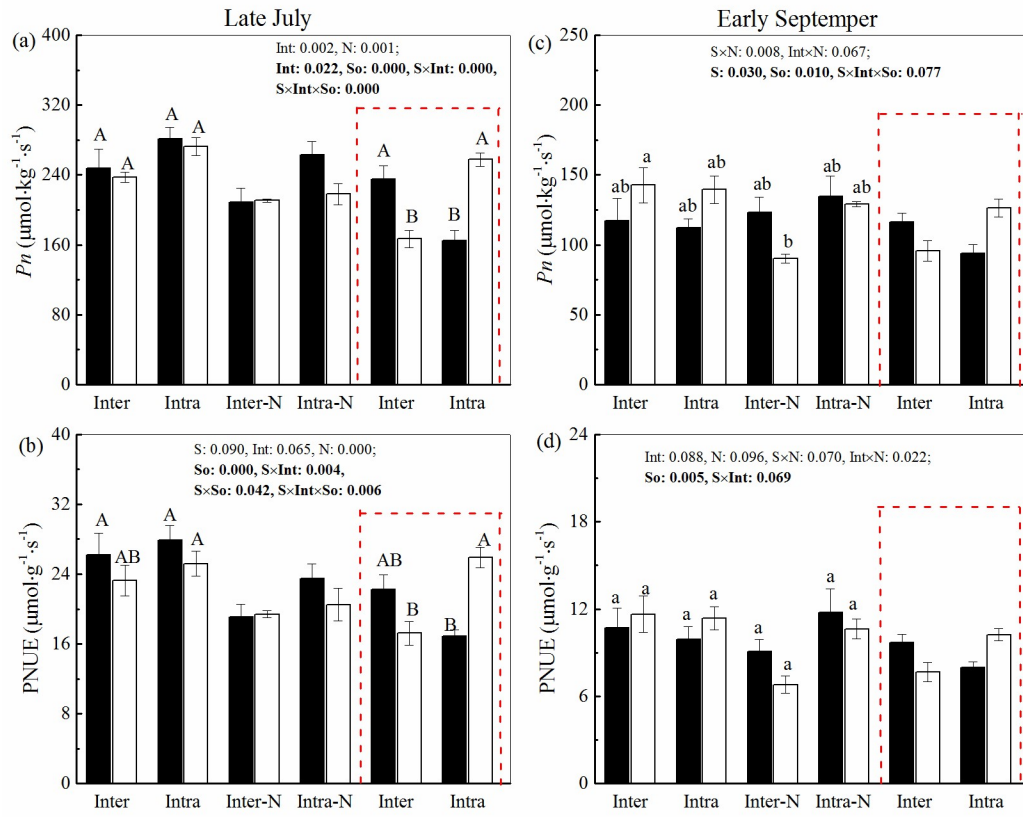
**Figure 7** Glutamate dehydrogenase (GDH) (a), glutamine synthetase (GS) (b) and nitrate reductase (NR) concentrations (c) in two *Larix* species in early September, 2015. GDH: Glutamate dehydrogenase, GS: glutamine synthetase, NR: nitrate reductase. Detailed information of symbols shown in **Fig. 1**.

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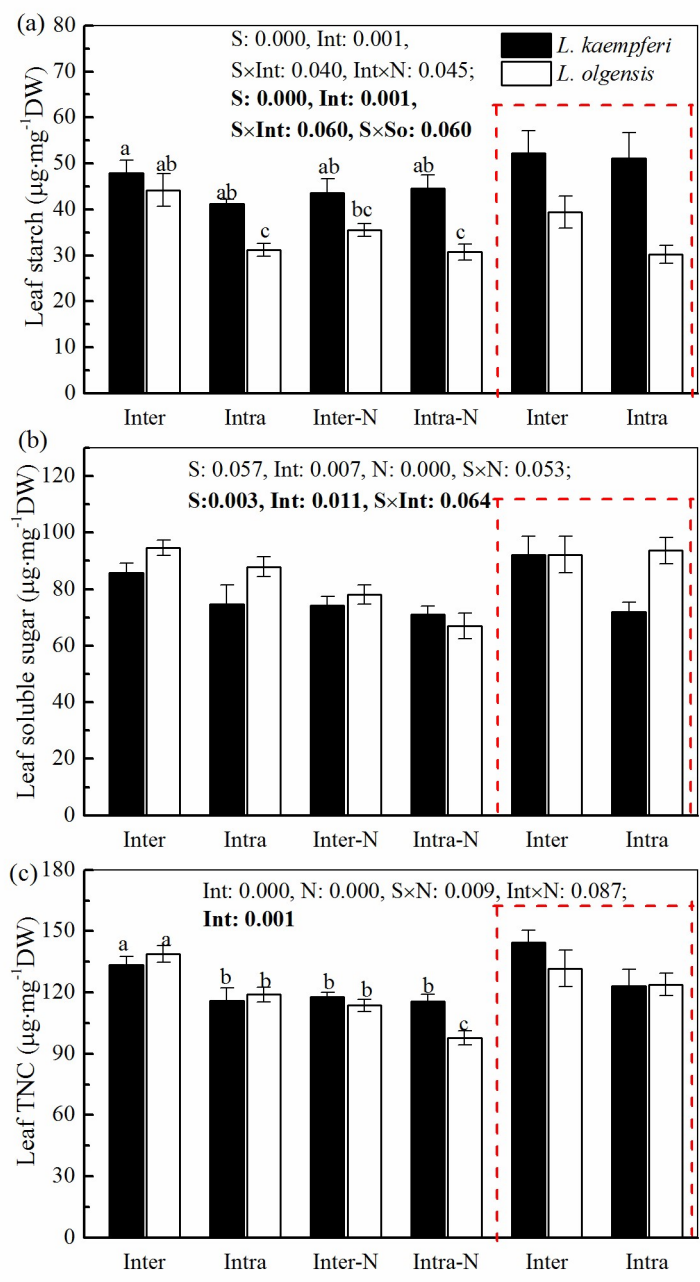
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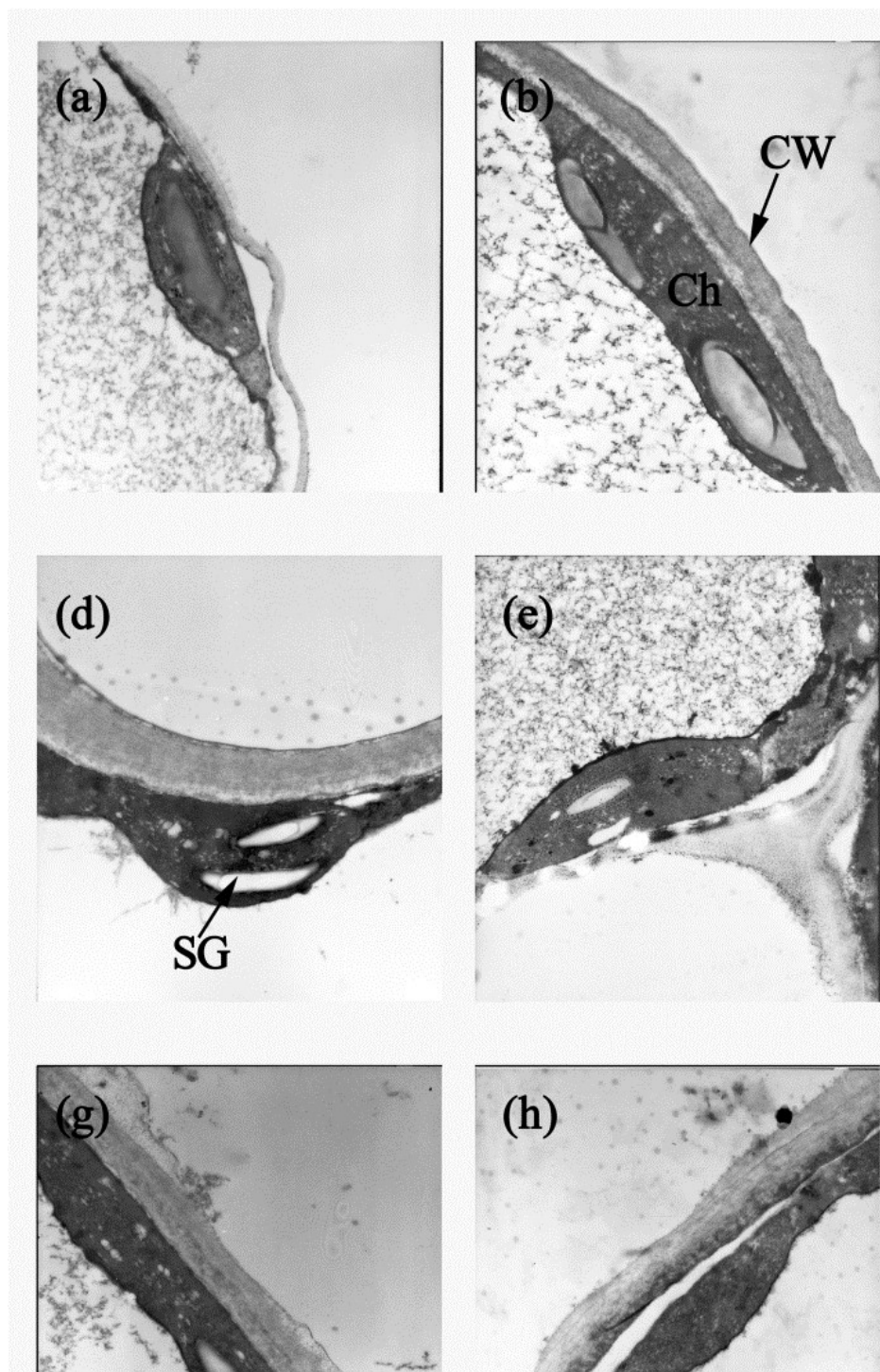
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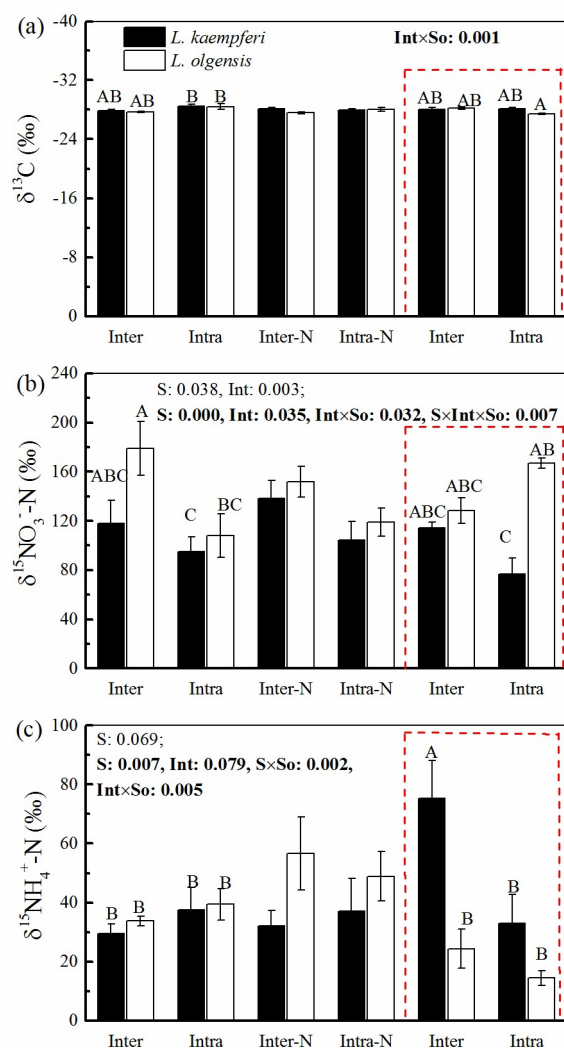


1 **Figure 4**  
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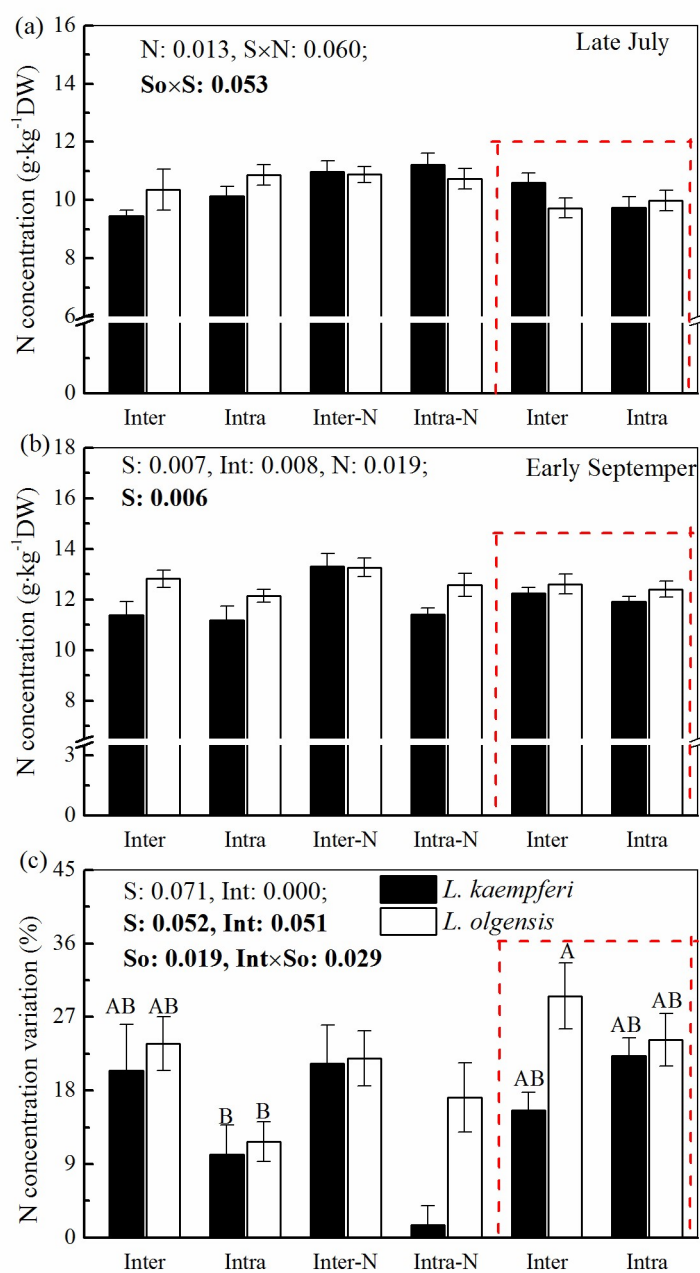
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1 **Figure 5**  
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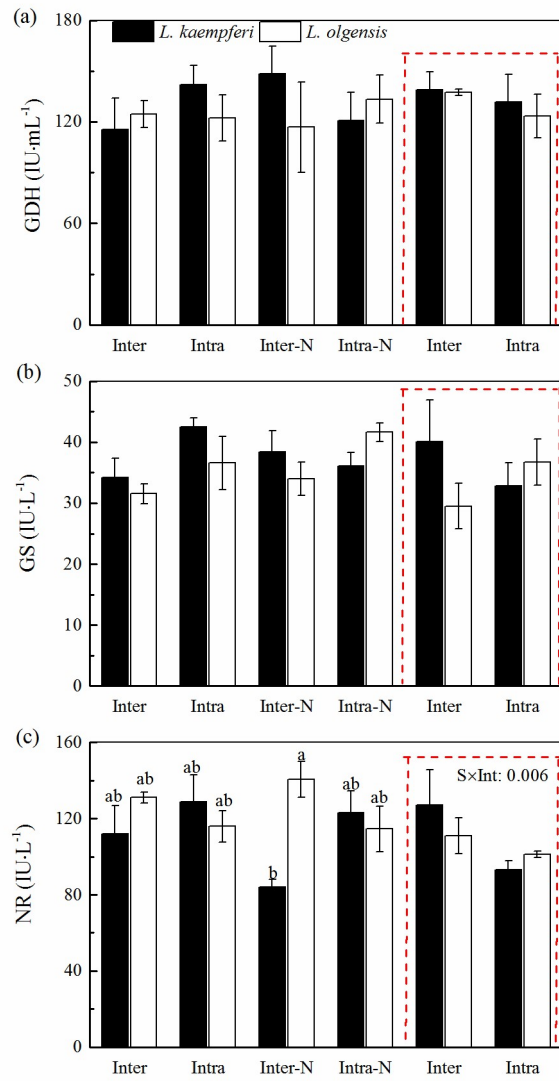
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1 **Figure 7**  
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